PB81-215923

Some Applications of Locomotor Response in Pollution Effects Monitoring

(U.S.) Environmental Research Lab.
Narragansett, RI

1980

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TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completi

1. REPORT NO.	2.	3.
EPA-600/J-80-287	Journal Article	PB81-215923

4. TITLE AND SUBTITLE	5. REPORT DATE
Some Applications of Locomotor Responses in Pollution Effects Monitoring	May 1981
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Don C. Miller	ERLN-J-158
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Environmental Research Laboratory South Ferry Road Narragansett, RI 02882	AAKN1C
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Environmental Research Laboratory South Ferry Road Narragansett, RI 02882	Journal Article
	14. SPONSORING AGENCY CODE
	EPA/600/05

15. SUPPLEMENTARY NOTES

Rapp. P.-r Réun. Cons. int. Explor. Mer, 179:154-161, 1980

16. ABSTRACT

The potential for behavioral tests in a pollutant effects aquatic monitoring program is discussed. Various types of individual and inter-individual behavioral responses are noted, but emphasis is placed on individual behaviors since they are of a less complex nature and may be easier to use routinely. Measurement of undirected (nonsimulated) locomotor activity may provide an index of neuromotor function. Locomotor responses to specific external stimuli may also be utilized to test specific motor and sensory capacities. Locomotor activity has been employed extensively in mammalian behaviorial toxicology; this parameter may also be valuable with aquatic organisms. This paper reviews past and some current research on the effects of pollutants on the locomotion in aquatic organisms, examining in some detail undirected activity, photoresponses, thermal regulatory responses and rheotaxis. Several criteria are cited to evaluate behavioral tests for monitoring purposes and some research needs in this area are enumerated.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

PRODUCT OF:
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to Public	Unclassified	7
	20. SECURITY CLASS *(This page)*	22. PRICE
	Unclassified	

EPA Form 2220-1 (9-73)

EPA-600/J-80-287
Journal Article

Rapp. P.-v. Réun. Cons. int. Explor. Mer, 179: 154–161. 1980.

SOME APPLICATIONS OF LOCOMOTOR RESPONSE IN POLLUTION EFFECTS MONITORING

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INTRODUCTION

The primary objective of pollution biomonitoring programs is to detect reduced water quality. It is additionally desirable to obtain an indication of the biological consequences of any degradation. At the species level, pollutants may be detected by monitoring sensitive life functions in well-studied test species. More difficult is the task of deducing the consequences of observed functional impairment for the survival of the organism or its population, much less extrapolating the effects to the larger marine community. Extensive research is required to resolve these latter questions. For the present, single species effects monitoring should focus on species of known ecological importance and utilize pollution stress indicators which can plausibly be related to success of the population in nature.

A biomonitoring program must utilize sublethal stress indices since pollutant concentrations in nature are typically very low, far below those causing conspicuous or catastrophic effects. The full spectrum of potential sublethal organism responses should be considered when selecting indices. Avoidance is one possible initial response. This requires motility and the capacity to perceive a pollutant. But if pollutant exposure continues, there is the potential for impairment of basic functional processes or control systems, with serious implications for: (1) behavioral response systems (which are dependent on normal function of the nervous, muscular, and possibly endocrine systems); (2) energetic processes (feeding, assimilation, respiration, and growth); (3) reproductive processes (gametogenesis, fertilization, and early development); and (4) morphological integrity (from cell ultrastructure to gross morphology). Impaired function or performance in any of these four general areas can then be utilized in pollution effects monitoring to signal the existence of a basic physiological problem. Sublethal pollutant effects have been reviewed by Sprague (1971) for fish, Rosenthal and Alderdice (1976) for marine fish eggs and larvae, and McIntyre et al. (1978) for marine animals generally. In addition to the four broad effects areas cited above, researchers have also considered the sublethal effects of pollutants on respiratory, circulatory, and renal biochemistry and physiology, immune response systems (as evidenced by incidence of disease and parasitism) and genetics.

Impairment of reproduction and early development are presently used routinely in bioassays as sublethal pollutant stress indices. The embryo-larval and early juvenile (fry) stages in fish are now known to be the most sensitive life stages, based on a large number of full life-cycle assays with various types of toxicants (McKim, 1977; Macek and Sleight, 1977). Accordingly, tests employing embryonic and larval material are frequently used in the USA to estimate maximal acceptable toxic concentrations (MATC), replacing full life-cycle chronic tests. Critical life-stage exposures for fish encompass the entire embryonic period, plus 30 to 60 days posthatch; pollutant effects indicators include egg fragility, hatchability, early growth, and survival.

Parameters of bioenergetics and growth are also currently recognized as highly promising pollutant stress indicators (McIntyre et al., 1978). Growth can be directly measured over short time periods in such organisms as protists and hydrozoans, as well as in the early life stages of higher animals. When growth cannot be readily measured, the energy available for growth can be estimated by measuring energy intake (feeding rate) and energy loss (via feces, excretion and respiration). Measurement of scope for growth can be quite useful, since pollutant effects on specific bioenergetic components of growth are identified.

Behavioral indices have been used in a limited manner in experimental assays (Olla, 1974), to monitor river water quality and to detect deleterious pollutants

Table 1. Behavioral responses potentially applicable in pollution effects testing (modified from Olla, 1974)

A. Individual responses	Parameters of measurement	B. Inter-individual responses
Locomotor responses		Predation efficiency/ vulnerability
Undirected locomotion (no experimental stimuli)	rate, pattern of movement, activity rhythms	Social interactions
Directed locomotion	sign, rate, pattern of movement, response threshold	aggregation
Test stimuli:		aggression
light		territoriality
temperature		
chemical		courtship
pheromone		
food		parental care
salinity		
pollutant		
currents	response threshold, motor endurance	
Other responses		
Learning		
Motivation		
Shelter building/shelter seeking		
Physiological rate-related activities	feeding, ventilation, heart rate	

in industrial effluents (Cairns et al., 1976). Fishes have been used primarily for monitoring; invertebrates could potentially also be employed. Where controlled laboratory tests can be incorporated into a pollutant effects monitoring program, behavior could be a very powerful pollutant indicator. Most aquatic organisms have evolved sensory systems capable of perceiving subtle environmental changes. Potentially, very low concentrations of pollutants may also be detected. Alternatively, behavioral tests can be utilized to signal pollutant-induced impairment of any component or process associated with a neuro-motor response system. Failure of a behavioral response system can lead to reduced fitness of individuals, with adverse consequences for the population.

The various types of behavioral responses one might consider for pollutant effects monitoring are listed in Table 1. The potential utility of individual locomotor responses for monitoring is the topic of this paper. Individual behaviors tend to be of a less complex nature than inter-individual responses and hence should be easier to use routinely. Controlled tests of undirected (i.e. free running) locomotor activity will provide one measure of nervous system function. Locomotor responses can also be utilized to test specific motor and sensory capacities with the use of appropriate external stimuli. Locomotor activity has been used extensively in mammalian behavioral toxicology and behavioral pharmacology, in part owing to its ease of measurement in small terrestrial mammals. It should be possible to do the same with aquatic animals, given existing electronic techniques to quantify locomotion. Kleerekoper (1976) has conducted pioneering studies of this type using fishes.

Closed-circuit television, mini-computers, and micro-processors are examples of readily available electronic tools which greatly facilitate behavioral data collection and analysis for effects monitoring. In the past, the time required to quantify observations and adequately replicate tests seriously limited the use of behavior in pollutant effects work. At our laboratory we have been able to reduce this time appreciably through computer analysis of videotaped behaviors. This approach also enhances test precision and accuracy of data analysis. A behavioral study can be conducted at any location and the responses videotaped. Computer analysis is basically a rapid frame-by-frame analysis of this record, with the x–y coordinates for all organisms in each video frame determined and stored in memory. Paths of travel are then plotted for the quantified video time series, from which parameters of motion are calculated, statistically evaluated, and summarized in numerical and graphical form. Our sample size is typically 15 to 20 animals per test. The system can be employed for mobility studies of organisms ranging in size from bacteria to fish. Descriptions of the system, examples of applications and the results of some pollution studies to date are included in papers by Greaves (1975), Wilson and Greaves (1979) and Lang et al. (1979a; b). A somewhat simpler system using a video camera and micro-processor has been developed by Lubinski

et al. (1977) to monitor the movement of single fish. Additional automation techniques for behavioral monitoring are cited in Cairns et al. (1976).

EFFECTS OF POLLUTANTS ON CERTAIN LOCOMOTOR ACTIVITIES

UNDIRECTED LOCOMOTOR ACTIVITY

Studies have been conducted to date on the effects of pesticides, metals, crude oil and dispersants on fish and invertebrate locomotion. A biphasic dosage response is often observed. At relatively low pollutant dosages, increased locomotor activity is frequently seen; those approaching the acutely lethal levels usually cause a decline. This generalization is illustrated by studies with bluegill sunfish (*Lepomis macrochirus*), using metals and DDT (Ellgaard et al., 1977; 1978). Swimming-rate constants were calculated after observing the dispersal of 40 fish in a semi-partitioned aquarium. Low metal and DDT concentrations elicited hyperactivity following three days of toxicant exposure. With cadmium, reduced activity was only seen in the 0·5 ppm treatment, a level which caused 30 % death in two weeks. DDT concentrations which approached the 96 LC_{50} initially caused hyperactivity, then suppressed activity by day 9.

The biphasic locomotor response to certain metals and pesticides has also been seen with invertebrates. Fingerman et al. (1979) observed a 91 % increase in undirected behavior, in fiddler crabs (*Uca pugilator*) following three days' exposure to 0·08 ppm DDT. Interestingly, the circadian component of their locomotor activity was not affected. This latter finding agrees with earlier work on this species by Vernberg et al. (1974) using mercury. These workers saw no change in locomotor rhythm in adults during a two-week exposure to 0·18 ppm, although this treatment suppressed activity. A 25 % reduction in swimming rate also occurred in larvae (stage I zoea) held for 6 h at the same concentration. All these larvae died within 24 h. In contrast, hyperactivity was seen in stage V zoeae which had been reared in lower concentrations of mercury (0·18 and 1·8 ppb). Hyperactivity has also been reported in crab larvae (*Rithropanopeus harrisii*) exposed to an insect juvenile hormone mimic and two insect growth regulators (Forward and Costlow, 1976; 1978). We have observed hyperactivity in juvenile mysids (*Mysidopsis bahia*) exposed to mercury (Fig. 1) and with barnacle larvae (*Balanus improvisus*) following exposure to low concentrations of copper. A high copper level (150 ppb) markedly suppressed barnacle larval locomotion within 24 h (Lang and Miller, unpublished).

Physiologically, pesticide-induced hyperactivity is believed to be the result of repetitive discharge of nerve impulses. This was shown for the lateral line nerve in brook trout following exposure to sublethal levels of DDT (Anderson, 1968). Fingerman et al. (1979) cite several workers who reported the same phenomenon with crustacean and cockroach nerves. Further research is required to determine if there is

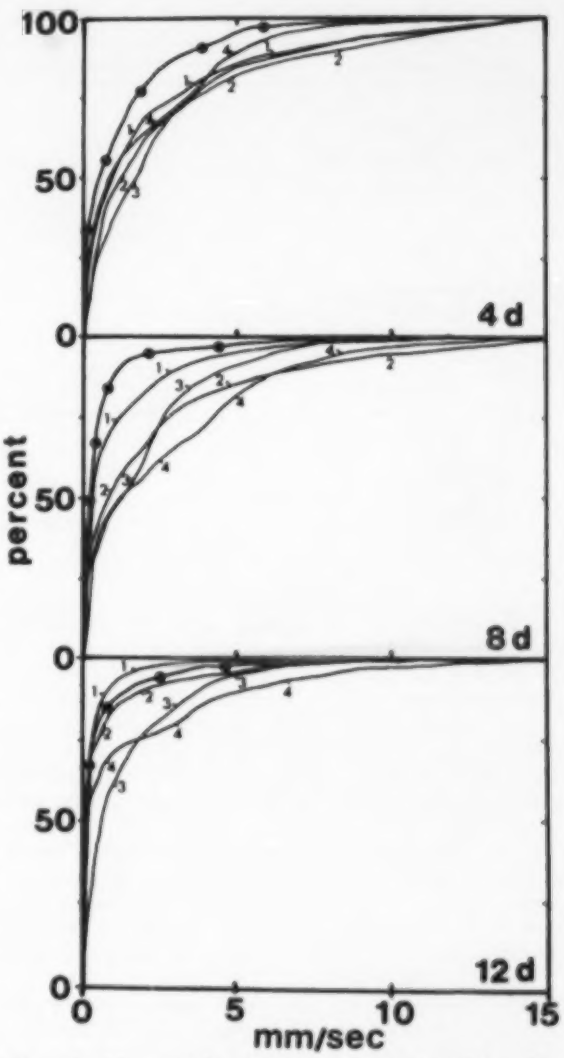

Figure 1. Mercury-induced hyperactivity in juvenile mysids (*Mysidopsis bahia*). $HgCl_2$ dose levels (ppb): one, 0·4; two, 0·7; three, 1·6; four, 2·3. Linear velocity is significantly different from controls ($P = 0·05$) on day 4 for level three; on day 8 for levels two, three, and four; and on day 12 for level three (Lang et al., in press).

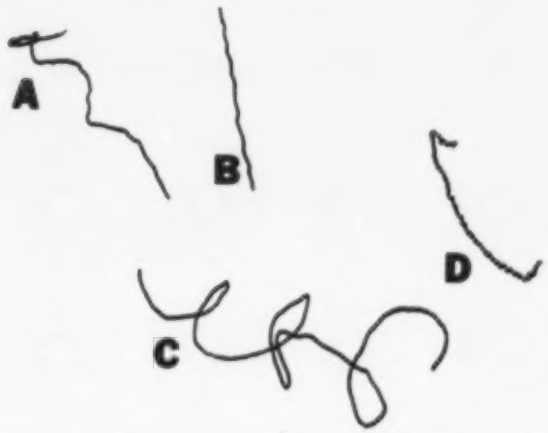

Figure 2. Examples of computer-tracked paths for stage II *Balanus improvisus* nauplii and assigned rate of change of direction values (degrees/s): (A) 114, (B) 53, (C) 152, (D) 373. Paths A, B are typical of control conditions; path C occurs more frequently with copper present; path D was observed only above 50 ppb copper (from Lang et al., 1979b).

a neurological basis for hyperactivity for other classes of pollutants.

A change in the spatial pattern of swimming can also be indicative of the presence of a pollutant. This was shown by Vernberg et al. (1974), with mercury inducing altered swimming in fiddler crab larvae from fairly linear patterns to increasingly circular paths. We have also observed changes in swimming pattern in barnacle nauplii exposed to copper (Fig. 2). Increased looping occurred at low dose levels, while a saw tooth pattern developed in some nauplii with 24 h exposures above 50 ppb. Shifts in swimming pattern could also be the consequence of hyperactivity *per se*, although the more aberrant patterns may signal neural disfunction. However, the total number of individuals of the test group ($n=40$) exhibiting altered swimming patterns was low. Increased linear velocity proved to be the more reliable indicator of low-level copper contamination.

POLLUTANT EFFECTS ON DIRECTED LOCOMOTOR RESPONSES

PHOTORESPONSES

The measurable parameters of photobehavior include the direction (sign) of orientation with respect to the light stimulus, the intensity of phototactic response (expressed as % of the test group responding) and swimming rate (photokinesis) following the stimulus. The light stimulus used should be of known spectral composition and intensity and be within the range normally experienced by the test organism. Often the wavelength and intensity selected will correspond to the organism's principal response maximum. However, use of a number of light intensities spanning the organism's normal range is initially recommended for pollutant effects testing. In the work with barnacle nauplii (*Balanus improvisus*, stage II), strong (50 %) sign reversal of phototaxis with 150 ppb copper was found at intermediate light intensities (Fig. 3). The effect was not evident at the high or low intensities tested. Subsequent tests were made at $4{\cdot}5\times 10^{-8}$ W/m^2, the intensity level which elicited the strongest positive phototaxis in the control nauplii.

Forward and Costlow (1976; 1978) used physiologically defined light stimuli in evaluating the effect of three insect growth inhibitors and a juvenile hormone mimic on crab larvae. They found a consistent reduction in the intensity of the positive phototaxis at low pollutant concentrations. High dose levels reduced the intensity of both positive and negative responses in the test group. Our studies with stage II barnacle nauplii show that moderate levels of copper (50 and 100 ppb) reduce the intensity of the response, but not the sign. At 150 ppb copper, sign reversal occurred (Fig. 3). Contrasting findings have been reported by Vernberg et al. (1973), with mercury acting to increase intensity of positive phototaxis in fiddler crab larvae. Such differences in photoresponse could result from differences in test methodology. In the latter study, high intensity white light was used as the stimulus. In a study with rock crab larvae (*Cancer irroratus*) and oil, Bigford (1977) also found reduction in the intensity of phototaxis at the lowest oil exposure level (0·1 ppm) and loss of this response in three of five larval stages at 1·0 ppm. Sign reversal was not evident.

Amiard (1976) has measured the effects of metals on photokinesis in first zoea for two species of crustaceans. His test metals (Co, Sr, Ag) influenced photostimulated swimming speeds in the same manner as described previously for undirected swimming. Hyperactivity occurred at the lower concentrations and reduced activity at high levels. Further work in this area is called for, however. We have not observed hyperactivity in our studies with copper when using a light stimulus which was behaviorally determined to be optimal for the barnacle nauplii. Rather, we found significant reductions in photokinesis at 40 ppb and above. Our copper exposures ranged from 20 to 150 ppb for 24- and 48-h periods.

The preceding studies show that photobehaviors are quite sensitive to certain pollutants, with low concentrations reducing the intensity of normal phototactic responses and, for copper at least, high doses capable of causing sign reversal. Either effect can be of considerable ecological significance for planktonic organ-

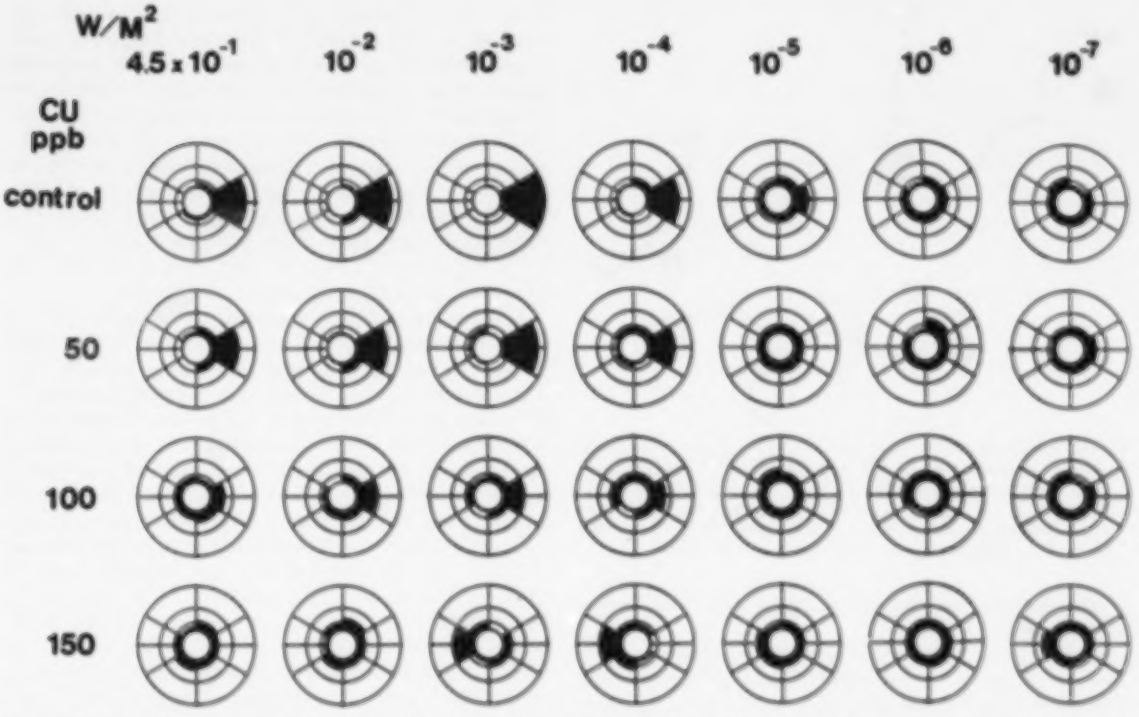

Figure 3. Directional response of stage II *Balanus improvisus* nauplii at 30‰ salinity after 24 h exposure without food to indicated concentrations of total copper. Polar histograms indicate per cent response of mean direction of travel for 28–60 nauplii at each condition during the latter 2·0 seconds of a 2·5 second 480 nm light stimulus of indicated intensity. A collimated light stimulus was presented at the right. Photoresponse directional intervals are ± 30° (from Lang et al., in press).

isms. Photoresponses are recognized as the primary behavioral responses involved in vertical migration of plankton, with the level of ambient illumination in the sea being the proximate stimulus. Vertical migration is a common planktonic phenomenon. Percy et al. (1977) observed diel migration of micronekton off the Oregon coast in 32 % of the larval fish species collected (including five of the seven most common species), 13 % of the shrimp and mysids and in 40 % of the cephalopods. The common migratory species usually occurred at depths of 300 to 500 m during the day and 0 to 50 m at night. Additional literature on diel migration of plankton and documentation of the major role of light stimuli is cited in recent reviews by Forward (1976) and Longhurst (1976). Vertical migration is generally believed to be adaptive for zooplankton by providing a zone of high-density food near the surface at night, then protection from predators as they move out of the euphotic zone during the day. It is certainly probable that reduced photoresponsiveness due to pollutants will impact population success among vertically migrating species.

In estuarine waters, light usually exists only at low levels, yet it is believed to function as one cue, probably secondarily to salinity, gravity and pressure, to influence the position larvae assume vertically in the water column (Sulkin, 1975). Appropriate water column position can be crucial for larval retention in an estuary. Forward (1977) has also suggested that a shadow response may exist in crustacean larvae which could provide protection from predators. In either case, shifts in the intensity or sign of phototaxis could potentially be deleterious for the survival of these organisms also.

THERMAL REGULATORY RESPONSES

Many fish behaviorally thermoregulate, swimming preferentially into waters having temperatures that most closely approach their optimum for metabolic function. The thermal preferendum can be determined with precision in the laboratory, using temperature-gradient tanks or electronic shuttle boxes. In the latter case, the movement of the fish between two thermally

distinct aquaria triggers heating or cooling, until the preferendum is reached (Neill et al., 1972). The preferendum is influenced by a number of biological variables, including recent thermal history. These must be taken into account in laboratory studies. The thermal regulatory mechanism in fish is believed to be similar to that of other vertebrates, with central nervous system control (Crawshaw, 1977).

The pollutants which have been shown to alter the thermal preferendum are primarily known neurotoxins or those with anesthetic properties. Effective compounds include organochlorine and organophosphorus pesticides (Ogilvie and Anderson, 1965; Gardner, 1973, Domanik and Zar, 1978). The anesthetic compounds include pentobarbitol (Ogilvie and Fryer, 1971), believed to interfere with the central control mechanism of the hypothalamus, and M.S. 222 (Goddard et al., 1974). The latter had a 3-day postexposure latent effect on lake trout. Anderson (1971) also found potassium cyanide (10 ppb) appreciably reduced the preferendum as well as metabolism in Atlantic salmon.

There is very good correlation between laboratory-determined thermal preferenda and the distribution of fish in nature. This was seen by early workers investigating the influences of physical factors on fish migration and more recently in many studies on the spatial distribution of fish in the heated effluents of power plants (e.g., Neill and Magnuson, 1974; Coutant, 1975). Pollutant-induced shifts in thermal response have the potential for reducing physiological efficiencies should fish lose their ability to locate thermally-optimal environments. Such shifts could also result in fish occupying habitats which overall are less suitable, placing them at a competitive disadvantage. Altered thermal behavior of anadromous fish could also impact timing of migratory runs.

RHEOTAXIS

Orientation and swimming can be induced in fish by creating a current. Accordingly, this stimulus has been employed to study the physiology of active fish and to evaluate fish condition by testing swimming endurance. Stamina testing can also be valuable to detect the presence or effects of pollutants. Active fish pass a high volume of water through the buccal cavity, maximizing exposure to pollutants (Besch et al., 1976). Reduced swimming performance in fish has been cited as a highly sensitive effects index for hydrogen sulfide (Oseid and Smith, 1972) and for bleached pulp mill effluent (Howard, 1975). Cripe (1979) found that impaired swimming ability also occurs in mysid shrimp (*Mysidopsis bahia*) when exposed to low levels of organophosphate insecticides. In one case, the effective concentration was equal to the level which impaired reproduction in a 28-day life-cycle test.

Swimming endurance measurement holds promise as a behavioral/physiological pollutant effects test. Stamina tests can be rapid indicators of stress since the organism engages in work while also experiencing maximal exposure to pollutants. Stamina tests permit evaluation of the functional integrity of a number of important physiological processes, namely respiratory gas exchange, energy mobilization, motor function and swimming coordination.

DISCUSSION

Behavioral tests should be considered as one of several types of stress indicators to be incorporated in a pollution monitoring program. Present evidence shows that behavioral response systems can be functionally impacted by low concentrations of certain pollutants. Indeed, the presence of neurotoxic contaminants may be sensed first by behavioral systems, either by avoidance or by impaired behavioral performance. Yet, it is also recognized that some pollutants will not affect the neuromotor system as a primary target; hence the need for different types of effects indicators when monitoring general water quality.

Three minimal criteria exist for the inclusion of a behavioral stress indicator in a pollution monitoring program. First, it should be practical to conduct the behavior test in a laboratory adjacent to the monitoring station. If this is not possible, the test species must be highly resistant to trauma from collection, handling, and transport. Behavioral assays normally require some control of environmental conditions which can only be provided in a laboratory setting. The specific requirements and design of a monitoring program will greatly influence the practicality of including laboratory assays in effects testing. As a second criteria, the behavioral test must be sensitive to at least one of the pollutants likely to occur at the locality of interest. Finally, the test must be practical to conduct in a routine manner. This may be facilitated in part by the development of electronic systems to automate data collection and analysis for behavioral tests of high promise.

Several types of locomotor responses have been advanced in this paper to detect the presence of pollutants and to document their effects on sensory or neuromotor systems. Chemosensory behaviors also hold real promise for monitoring certain pollutants (Sutterlin, 1974; Olla et al., 1980). Pollutant avoidance responses, for example, often are the result of chemically mediated detection. Also, pollutant masking or damage

to chemosensory systems can be tested by measuring an organism's ability to detect and move towards food, to respond to sex pheromones or to retreat in the presence of alarm substances. The relative sensitivity of all such behavioral systems should now be documented quantitatively and, by using a broad spectrum of pollutant types, qualitatively. Such comparative studies obviously must be well controlled for the major biotic and abiotic variables which influence each response. Further, in studies of directed responses, the test stimuli employed must be quantitatively and qualitatively within the range normally experienced by the organism in nature. The importance of this has been shown here for photobehaviors. The same is known to apply to chemically mediated response systems.

Comparative research on response sensitivity should permit identification of behavioral tests of highest promise for pollutant effects monitoring. Next, these tests must be compared with other existing sublethal pollutant stress indicators, evaluating their utility for monitoring from the standpoints of the concentration threshold, speed of the response, relative sensitivity to contrasting types of pollutants and overall ease of use. Sensitivity can be numerically described by the ratio of the effective dose for a sublethal response to an acutely lethal dose. In work on pulp and paper mill effluents, for example, thresholds for the more sensitive sublethal tests have been found to be 0·006 to 0·33 of the 96-h LD_{50} (Davis, 1976; Walden, 1976). Sensitivity expressions should be expanded to also indicate the time required to induce a sublethal response.

Research on the question of the biological and ecological significance of a behavioral test should also be pursued for those tests showing highest sensitivity and utility for monitoring. The nature, occurrence and role of relevant behaviors must be well understood in the context of the organism's overall adaptive strategy. Extensive field investigation is required to obtain this information. An understanding of the physiological basis of observed behavioral impairment is also important in order to predict the more plausible implications of pollution for the biota.

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